Proc. Natl. Acad. Sci. USA
Vol. 78, No. 5, pp. 3083–3087, May 1981
Developmental Biology

Regulation of synthesis of cell-specific ribosomal proteins during differentiation of *Dictyostelium discoideum**

(slime mold/development/ribosome heterogeneity/ribosomal proteins/translational control)

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Communicated by B. L. Horecker, February 2, 1981

ABSTRACT **Proteins of ribosomes from various stages of development in *Dictyostelium discoideum* were analyzed by two-dimensional polyacrylamide gel electrophoresis. Significant changes in protein composition were observed; the data demonstrate that cell differentiation in a eukaryotic system is accompanied by ribosome heterogeneity. Both qualitative and quantitative differences were noted for 12 unique ribosomal proteins between the vegetative amoebae and spores (differentiated cells). Two proteins were specific to ribosomes of amoebae, and three were specific to spores. The others were common to both cells but showed characteristic stoichiometric changes. The appearance and quantitative changes of these proteins were associated with specific stages of cell differentiation and were evident only during the aggregation phase; however, further changes continued through construction of fruiting bodies. As functional mRNAs for all 12 proteins were present in both amoebae and spores, both transcriptional and translational mechanisms apparently regulate the synthesis of the various developmentally controlled ribosomal proteins in the two cell types.**

Although our knowledge of the eukaryotic ribosome is incomplete, the evidence accumulated so far suggests that it is much more complex than the prokaryotic ribosome (1–3). A typical eukaryotic ribosome is composed of four RNA species and 70–90 proteins. Although ribosomes in all cells appear to perform the same function, it is not clear why more complex structures are assembled in eukaryotes than in prokaryotes. Therefore, detailed studies are warranted on the assembly of the eukaryotic ribosome and on the functions of its individual components, in particular, the role of the numerous proteins.

During differentiation in eukaryotes, the rate of protein synthesis and the types of proteins made may be different in the various cell types. A number of mechanisms may control protein synthesis during development. Cell-specific ribosome populations that vary in protein content might be responsible for regulating tissue-specific protein synthesis (4). However, previous searches for cell- or tissue-specific ribosomal proteins in different eukaryotic systems (5–8) have been inconclusive.

In the present study, we analyzed the composition of the various populations of ribosomes from *Dictyostelium discoideum* at different stages of cell differentiation. Ribosomal proteins of this organism are similar to those of other higher eukaryotic cells (9) and the system offers advantages not possible in most eukaryotes (5–8). It has a short generation time (≈8 hr), the cells develop synchronously and, within 24 hr, the developmental cycle is complete, and developmental mutants are easily obtained (10). The data establish that in the slime mold, (*i*) distinct differences exist in a set of specific ribosomal proteins between vegetative cells and differentiated spores and (*ii*) the synthesis of these proteins is regulated during specific stages of the developmental cycle.

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MATERIALS AND METHODS

Amoebae and Spores. *D. discoideum*, strain Ax3, was grown axenically in HL5 medium (9), and the amoebae were harvested during the logarithmic growth phase. The vegetative amoebae were washed once with 0.2% NaCl and stored frozen. Spores were prepared as described (11) and stored in 10 mM potassium phosphate buffer, pH 6.7/20% (vol/vol) glycerol at −25°C.

Development. Exponentially growing cells during their third or fourth passage in HL5 medium were routinely used. Cultures at a density of 6–7 × 10^6 amoebae per ml were harvested, washed twice in 10 ml of cold MES-LPS solution [1.5 g of 2-morpholinoethanesulfonic acid/1.5 g of KCl/0.6 g of $MgSO_4$/0.5 g of streptomycin sulfate per liter (pH 6.5)], and suspended at 1.8 × 10^8 cells per ml in the same solution. Filters for development of cells were prepared in 60 × 15 mm Petri dishes (12). The lower half of the Petri dish contained a circle of Whatman 50 paper resting on a pad of Whatman 17 paper, both of which had been soaked in MES-LPS solution. The cell suspension (0.4–0.5 ml) was spread uniformly on the filter and allowed to soak for 5 min, and then the excess liquid was removed. The upper half of the Petri dish contained a pad of Whatman 17 paper prewetted with 1 M Na_2KPO_4/1 M KH_2PO_4 (1:5), pH 6. The cells were allowed to develop in the dark at 24°C. Fruiting bodies were constructed within 24 hr. Developing cells were harvested at various times of incubation and washed once with 0.2% NaCl.

Ribosomes and Ribosomal Proteins. Amoebae, spores, or developing cells were sonicated in 20 mM Tris·HCl, pH 7.8/20 mM KCl/10 mM $Mg(OAc)_2$/5 mM 2-mercaptoethanol/5% sucrose and treated with 0.5% Nonidet P-40, and a postmitochondrial supernatant was prepared (9). A total ribosomal pellet was obtained by layering the supernatant on a cushion of 32% sucrose in the above buffer and centrifuging for 3 hr at 60,000 rpm in a Spinco 65 rotor. Proteins were extracted from the ribosomes with acetic acid and processed for electrophoresis as detailed (9).

Electrophoresis. Proteins from the ribosomes were analyzed on two-dimensional polyacrylamide gels (9). The first dimension gel was 8.0 M urea/59 mM bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bistris)·HOAC, pH 4.5/4% (wt/vol) acrylamide/0.1% methylenebisacrylamide, and the second dimension gel was 0.143 M Bistris·HCl, pH 6.75/10% acrylamide/0.5% methylenebisacrylamide/0.2% $NaDodSO_4$.

* This is paper No. 2 in the series, "Studies on Ribosomal Proteins in *Dictyostelium discoideum*." Paper No. 1 is reference 9.

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RESULTS

Comparison of Ribosomal Proteins from Amoebae and Spores. Ribosomal proteins were prepared from two stages of the slime mold life cycle, the undifferentiated amoeba and the differentiated spore. The patterns of the 80S ribosomal proteins are shown in Fig. 1. A majority of the proteins, including the large acidic proteins in the top left corner of the electropherograms, were identical in the two cell types. However, 12 proteins showed distinct qualitative and quantitative differences.

Two classes of proteins were distinguished: Members of the first class were in significant quantities associated exclusively with one or the other cell type (Table 1). Thus, proteins B, H, J, and K were present only in vegetative amoebae, and proteins A, D, E, and L were present only in the spores. Occasionally, protein D was observed in amoebae, and proteins H and J were observed in spores, but only trace amounts were detectable in these instances. Members of the second class were associated with both cell types but in significantly different amounts. The relative amounts of proteins F and G were higher in amoebae (see Fig. 3A) and those of C and I were higher in spores. The above differences were found in unwashed ribosomes (Fig. 1 *Left*) and in ribosomes washed with high-salt (1 M KCl) solution to eliminate loosely bound nonribosomal proteins (see Fig. 1 *Right*). Only protein A was lost easily on salt washing (0.5 or 1 M KCl). This indicates that these proteins are an integral part of the slime mold ribosome. As changes in proteolytic activities during morphogenesis may lead to differences in ribosomal proteins (13), the protease inhibitor phenylmethylsulfonyl fluoride (1 mM) was included during the isolation of ribosomes and the extraction of proteins from the amoebae and spores. No differences were detectable with and without the inhibitor (data not shown).

Ribosomes from amoebae and spores were dissociated into subunits to localize the various proteins designated as A–L in Fig. 1. Dissociation leads to a loss of protein A from spores and of protein D from amoebae but not spores (data not shown). Except for protein A, all the other proteins have been assigned either to the small or large ribosomal subunit. Proteins B–H belong to the small subunit and I–L to the large subunit. These proteins are unique in their electrophoretic behavior and molecular weight (see Table 1). We consider that protein A is a ribosomal protein that occurs in the undissociated monosomes, as none of the protein is present in the postribosomal supernatant (not shown).

Changes in Cell-Specific Proteins During Spore Germination. Germination of dormant spores into amoebae occurs in buffer, and the emerged amoebae do not undergo cell division. The entire sequence of spore germination is complete by 4 hr (11), and the early events of differentiation of the spores can be rapidly monitored. Fig. 2 shows the electropherograms of ribosomal proteins from cells harvested after 1.5 and 4.5 hr of activation of the spores. The pattern at 1.5 hr resembled that

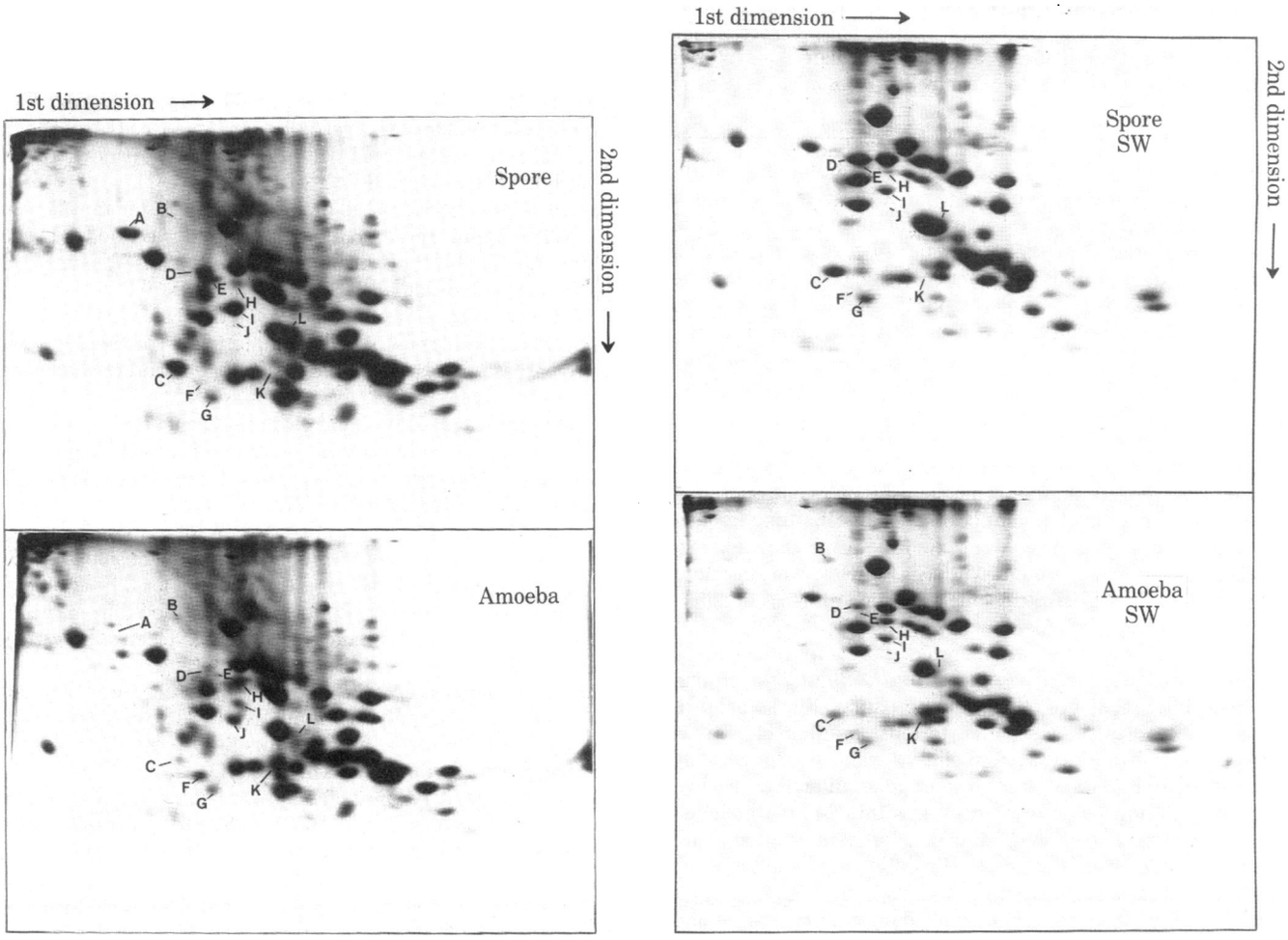

FIG. 1. Comparison of ribosomal proteins from amoebae and spores. (*Left*) Ribosomes from amoebae and spores were prepared as described in *Materials and Methods*. Proteins from 10 A_{260} units of ribosomes from each cell type were subjected to electrophoresis and stained with Coomassie blue. The cell-specific proteins are designated A–I. (*Right*) The same ribosomes after washing with 1.0 M KCl (9).

Table 1. Differences in proteins of ribosomes from amoebae and spores

Protein	M_r ($\times 10^{-3}$)	Identification	Relative abundance	
			Amoeba	Spore
A	43.8	80S	−	+++
B	49.5	S5	+	−
C	19.4	S6	+	+++
D	35.0	40S	±	++
E	34.2	40S	−	++
F	17.4	S10	++	±
G	16.1	S14	+++	+
H	31.7	S16	++	±
I	27.5	L10	+	+++
J	24.8	L11	+++	±
K	17.7	L18	+	−
L	24.5	60S	−	+++

Ribosomal proteins from amoebae and spores were separated as described in the legend to Fig. 1. For proteins appearing in both amoebae and spores, + denotes low and ++ or +++ denote larger amounts; − denotes the absence, and ± denotes a variable amount of a protein. The molecular weights of the proteins were determined in $NaDodSO_4$ gels as before (9). The identification of the proteins is based on analysis of the 80S ribosomes and the dissociated ribosomal subunits. S and L refer to small and large subunits as proposed (9). Those proteins denoted 40S or 60S are known to be associated with the respective ribosomal subunits, but no numerical designation has been given to them.

of the unactivated spores (see Fig. 1) except that there was a small decrease in the intensity of protein A and an increase in the intensities of the proteins immediately to the left and right of A. Although no obvious changes in the other proteins were apparent until 3 hr (unpublished results), within the next hour, dramatic changes in most cell-specific proteins occurred. The spore-specific proteins A, E, and L completely disappeared and proteins B, H, J, and K which were specific for the amoebae, appeared (Fig. 2*B*). The content of other cell-specific ribosomal proteins was also altered, but no changes were obvious in the remainder of the proteins.

Sequential Changes in Cell-Specific Ribosomal Proteins During Development. Although the emergence of amoebae from spores takes only a short time, the development of amoebae into spores involves relatively longer times. If cells from a growing culture are plated on filters, they aggregate and develop synchronously (12). The fruiting bodies are formed within 24 hr. An analysis of the ribosomal proteins from cells harvested at various stages of development showed that significant changes occurred at 6, 18, and 23 hr after plating the cells. These changes are summarized in Fig. 3.

During logarithmic growth, the various ribosomal proteins occurred in the relative amounts shown in Fig. 3*A*. This pattern was maintained for a majority of the proteins even in the early aggregation phase. However, four proteins (F, G, I, and J) showed dramatic changes during this early period of cell development (Fig. 3*B*). Proteins G and J, which were present in large amounts in logarithmically growing cells, decreased in concentration during aggregation. The relative amounts of proteins F and I increased at the same time. In some preparations of logarithmically growing cells, protein C was difficult to see in gels but always became quite distinct during early development. Furthermore, a trace of protein D appeared during the late aggregation phase (not shown).

At 18 hr of development, when pseudoplasmodia are present, three of the spore-specific proteins—A, E, and L (see Fig. 1 *Left*) appeared, and two proteins—B and K—specific for the amoebae disappeared (Fig. 3*C*). Also, quantitative changes

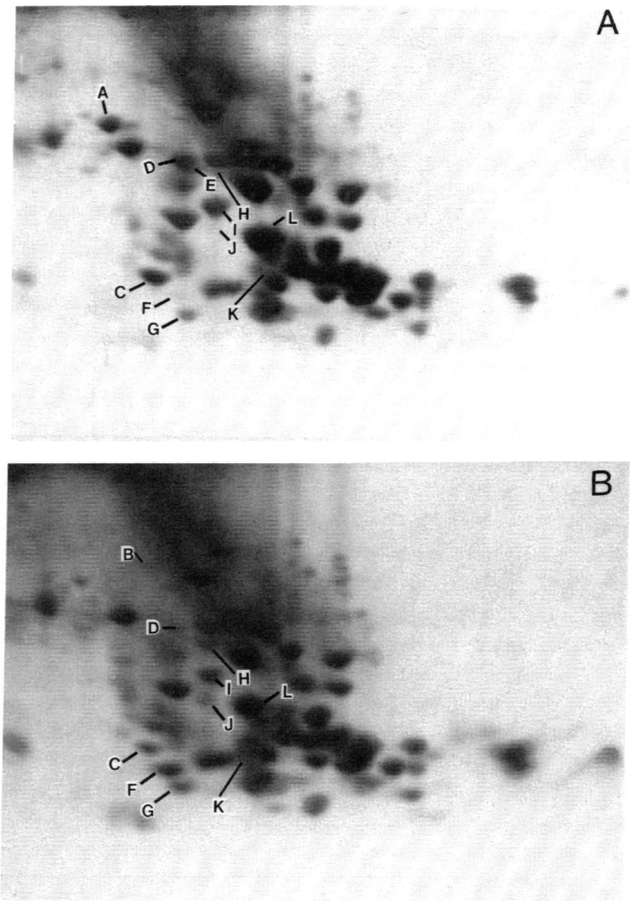

FIG. 2. Changes in cell-specific proteins during spore germination. The spores were suspended in 10 mM potassium phosphate buffer, pH 6.7/20% dimethyl sulfoxide and incubated for 30 min at 24°C. The activated spores were freed of dimethyl sulfoxide and then incubated in potassium phosphate buffer for 1.5 hr (*A*) and 4.5 hr (*B*) and harvested. Ribosomal proteins were analyzed as in Fig. 1. The pattern of proteins in the 0-hr unactivated spore is shown in Fig. 1 *Left*.

were apparent in other cell-specific ribosomal proteins. Proteins C and D increased significantly and proteins H and J were reduced. In the next phases of development, culmination and spore formation, only further quantitative changes occurred in these proteins. The amounts of proteins A, C, E, I, and L increased enormously and those of F, H, and J were reduced to traces (Fig. 3*D*). In this figure, proteins L41, S33, and S34 (ref. 9; arrow in Fig. 3*A*) are decreased in intensity. This was not observed in other preparations. Table 2 summarizes the pattern of synthesis and accumulation of the 12 cell-specific ribosomal proteins during growth and differentiation.

DISCUSSION

A simple yet appealing hypothesis to explain the changing rates of protein synthesis and synthesis of tissue-specific proteins in differentiating organisms is to assume a switch in ribosome population. This model is especially meaningful as more and more recent studies on ribosomes have discovered specific roles for its individual components (2, 14). Some evidence for a subpopulation of ribosomes in *Escherichia coli* has been provided (15). However, no convincing evidence for the heterogeneity of ribosomes has so far been found in the several animal cells studied (5–8). One of the significant aspects of the present study is that our findings provide firm evidence for distinct populations of ribosomes during cell differentiation in a eukaryotic system. We

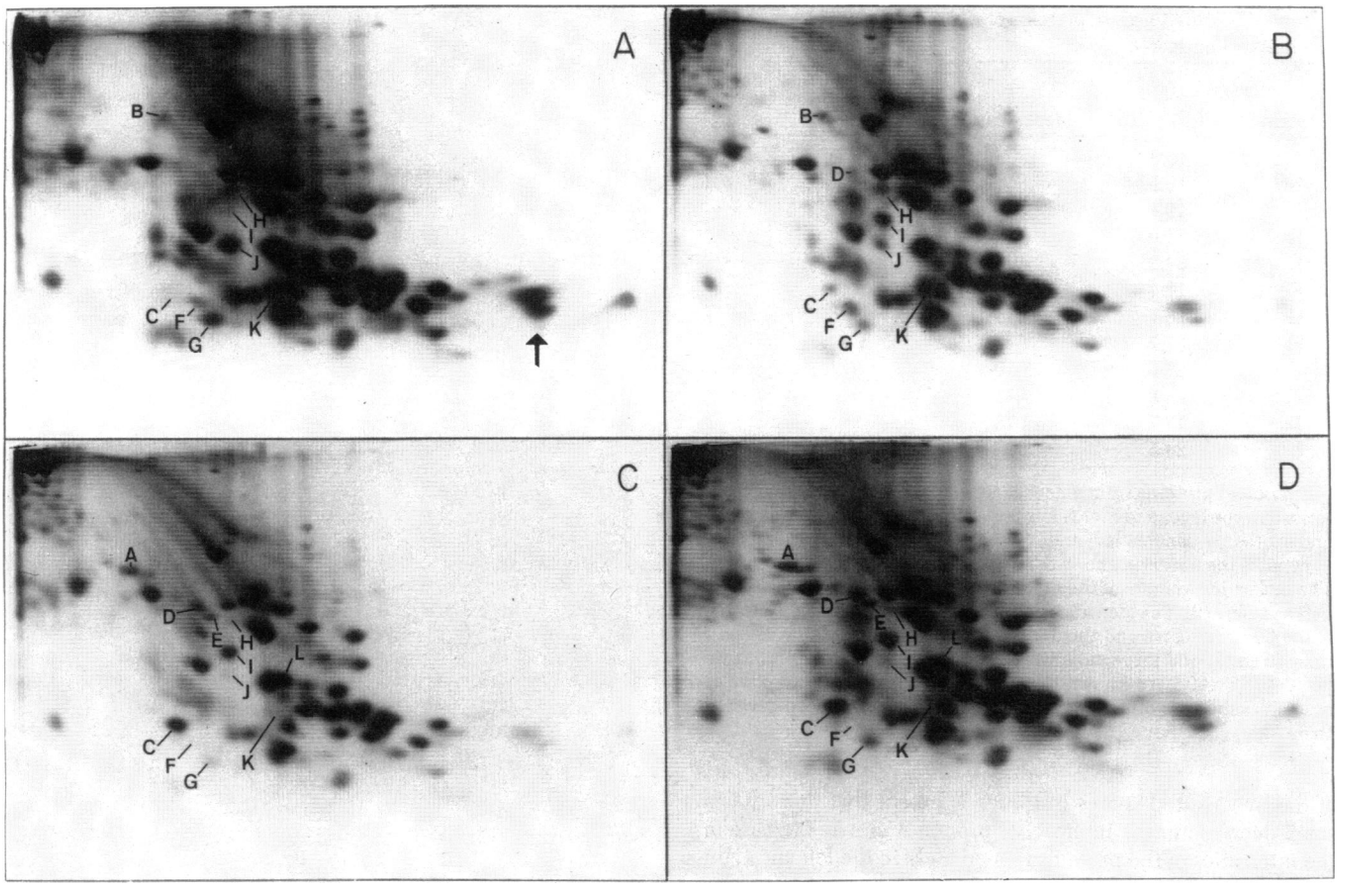

FIG. 3. Comparison of ribosomal proteins from growing and developing cells of *D. discoideum*. Vegetatively growing amoebae and the developing cells were prepared as described in *Materials and Methods*. Proteins from 14 A_{260} units of ribosomes were separated as described in the legend to Fig. 1. (*A*) Vegetative amoebae (0-hr cells). (*B*) Aggregation phase (6 hr). (*C*) Pseudoplasmodia-culmination phase (18 hr). (*D*) Fruiting body (23 hr).

have found that this heterogeneity is shown by differences in a specific set of ribosomal proteins (see Fig. 1 and Table 1) and not by rRNA; the latter remained invariant during development (ref. 16; unpublished data) as analyzed in sucrose density gradients and by hybridization competition experiments. Our results establish that authentic ribosomal proteins exhibit both cell-specific distribution and modulation during specific stages of development. Previous studies (17, 18) have not adequately established the role of development in the regulation of ribosomal proteins. and the present data may provide a basis to elucidate the asssembly and functional aspects of complex eukaryotic ribosomes.

It is noteworthy that Cocucci and Sussman (19) found that ≈75% of the ribosomes made during exponential growth disappear during subsequent development and are replaced by new ones synthesized during the morphogenetic sequence. This result, in conjunction with our present findings concerning the changes in distribution of ribosomal proteins during development, suggests an intriguing explanation. It is possible that the new ribosomes synthesized during fruiting body formation represent a new type of ribosome that is functionally different from ribosomes of the growing cell stage.

We showed that 12 ribosomal proteins are subject to regulation during slime mold development. These constitute a significant fraction, ≈15%, of the total ribosomal proteins established in this organism (9). Several biochemical criteria establish that these proteins are *bona fide* components of the ribosomes.

Slime mold ribosomes are exceptionally sensitive to treatment with high-salt solution and unfold during such treatment, resulting in some loss of proteins. However, the majority of the proteins found in low-salt solution-washed ribosomes remain even after extensive washing with up to 1.5 M KCl. None of the

Table 2. Stage-specific synthesis of ribosomal proteins during slime mold development

Stage	Relative amount of protein											
	A	B	C	D	E	F	G	H	I	J	K	L
Growth	−	+	+	±	−	++	+++	++	+	+++	+	−
Aggregation	−	+	+	±	−	+++	+	++	++	++	+	−
Pseudoplasmodium	+	−	++	++	+	++	+	+	++	+	−	+
Culmination	+++	−	+++	++	++	+	+	+	+++	±	−	+++
Fruiting body	+++	−	+++	++	++	±	+	±	+++	±	−	+++

Ribosomes were prepared from logarithmically growing cells and from developing cells as described in *Materials and Methods*. Equal amounts of proteins were separated as in Fig. 3. The morphological stages of development shown are approximate. Presence, absence, and relative amounts of protein during development were estimated by Coomassie blue staining. −, absent; ±, variable; +, low; ++, high; +++, higher.

12 proteins we are considering, except protein A, is washed off the ribosome particle by high-salt solution. This was also done by using isolated 40S and 60S ribosomes. Furthermore, we could not find any of these ribosomal proteins in the postribosomal supernatant. We therefore feel that these criteria allow us to conclude that these are *bona fide* ribosomal proteins.

The data suggest a complex pattern of regulation of the various cell-specific ribosomal proteins during development. The regulation of the 12 proteins falls into five separate classes (see Table 2): (*i*) proteins such as A, E, and L that are absent in ribosomes of growing and aggregating cells appear in cells during late development; (*ii*) proteins B and K disappear from late development ribosomes; (*iii*) there is a progressive increase in the amounts of proteins C, D, and I during development; (*iv*) there is a progressive decrease in the amounts of proteins G, H, and J during development; and (*v*) protein F is present throughout growth and development but its relative concentration increases only during aggregation. Furthermore, there are certain quantitative differences among the proteins in each class. Most changes apparently occur after cell contact, during the late aggregation-pseudoplasmodium stages, but significant changes also occur at later stages of development.

The influence of the various cell-specific proteins on the three-dimensional structure of ribosomes from amoebae and spores was evaluated in a recent study (20). The high-resolution electron microscopic images of the two types of ribosomes showed no detectable differences. However, an analysis of the secondary structure of RNA in ribosomes by circular dichroism and thermal melting indicated a higher stability of spore ribosomes. These results indirectly suggest that the interaction of spore-specific ribosomal proteins with RNA may contribute to this effect. In addition to ensuring the stability of ribosomes, some of these proteins might also be involved in the regulation of protein synthesis during growth and development. A possible function would be mRNA selection and translation of cell-specific polypeptides.

Our description of the 12 developmentally regulated proteins as the structural proteins of the ribosomes expands the list of known proteins for subsequent studies on the regulation of protein synthesis during development. These important proteins would have escaped the detection methods used by other investigators (21–24).

In another investigation (unpublished results), we found that translatable mRNA was present for all 12 proteins in both amoebae and spores. The regulation of the cell-specific ribosomal proteins appears to be different from that of other developmentally regulated proteins studied in this organism. Most proteins are apparently transcriptionally controlled (21–23, 25, 26). In contrast to these proteins, the amoebae-specific ribosomal proteins are apparently regulated by both transcriptional and post-transcriptional mechanisms during logarithmic growth. Vegetative amoebae lack proteins A, E, and L (see Fig. 1 *Right*) yet contain mRNA for these proteins that can be translated *in vitro* (unpublished results). As only mature ribosomal particles were analyzed, one might suppose the missing proteins to be present elsewhere in the cells. However, there was no evidence for the presence of these proteins in the postribosomal supernatant. These factors, therefore, suggest that some of the amoebae-specific ribosomal proteins may be regulated by translational mechanisms.

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